

NATURAL HYBRIDIZATION IN THE GENUS *CIRSIUM*:
C. ALTISSIMUM × *C. DISCOLOR*—CYTOLOGICAL AND
MORPHOLOGICAL EVIDENCE

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ABSTRACT. Cytological and morphological evidence indicates natural hybridization between *Cirsium altissimum* and *C. discolor*. It is postulated that the high degree of hybrid sterility is correlated with meiotic abnormalities.

Key Words: *Cirsium altissimum*, *Cirsium discolor*, hybridization, hybrid sterility, Nebraska.

In the genus *Cirsium* (family Asteraceae, tribe Cardueae), variability and intergradation of diagnostic characters make morphological circumscription of many species challenging and difficult. The difficulty is partly explained by the occasional breakdown of sterility barriers between closely related species, thus permitting interspecific hybridization (Ownbey 1951, 1964; Davidson 1963; Bloom 1977; Dabydeen 1987).

Cirsium altissimum (L.) Spreng. and *C. discolor* (Muhl.) Spreng. are two biennial, sympatric species sharing similar mesophytic habitats within an extensive eastern North American range. Both species have a similar extended flowering phenology; thus reproductive structures at all stages of development ranging from immature floral buds to mature capitula are usually observed simultaneously on the same plant. It is generally believed that members of the genus *Cirsium* employ both autogamous and allogamous reproductive strategies.

Petrak (1917) placed *Cirsium altissimum* and *C. discolor* in his series *Altissima* to reflect close affinity. A closer relationship is seen in Gray's (1884) treatment of *discolor* as a variety of *C. altissimum* (as *Cnicus altissimum*).

Phenotypically, *Cirsium altissimum* and *C. discolor* are easily distinguishable and are separated primarily by the degree of lobing or indentation of the leaves. According to Frankton and Moore (1963), in *C. discolor* the "cauline leaves are deeply pinnatifid into narrow linear-lanceolate lobes," and in *C. altissimum*

the "cauline leaves are entire or subentire with shallowly sinuate lobes."

Cytologically, in *Cirsium altissimum* and *C. discolor* chromosome numbers have become extremely reliable in the determination of the species, even though supernumerary chromosomes have been observed infrequently in *C. discolor* (Frankton and Moore 1963; Ownbey and Hsi 1963; Bloom 1977).

Frankton and Moore (1963) measured somatic chromosome lengths of *Cirsium discolor* ($2n = 20$) and *C. altissimum* ($2n = 18$) and found no significant differences between the total chromatin lengths of these taxa nor in the size ranges of the individual chromosomes. Apparently, both genotypes contain the same amount of material. They argued that the reduction in the chromosome number of *C. altissimum* may have been accomplished by translocation of portions of two chromosomes to the eighteen surviving chromosomes.

Bloom (1977) mentioned that his results of initial studies (unpublished) of *Cirsium altissimum* and *C. discolor* hybrids showed that chromosomal arrangement of these two species differ by a minimum of four reciprocal translocations and one paracentric inversion.

Observation of a *Cirsium* plant with an unusual leaf lobing pattern (in Nebraska just across the Missouri River from a mixed population of *C. altissimum* and *C. discolor* plants in Iowa) prompted this investigation. The mixed population was growing in an area approximately thirty by ten feet on top of the Missouri River bank. The area was lightly vegetated, mostly with small shrubs and herbs. Beyond the riverbank patches of tall shrubs and a few small trees dominated in what appeared to be abandoned pasture lands.

In *Cirsium altissimum* and *C. discolor*, reports based on analyses of morphological variation have revealed evidence of natural hybridization (Davidson 1963; Ownbey 1964). To this author's knowledge, no cytological evidence supporting spontaneous hybridization between *C. altissimum* and *C. discolor* exists in the literature. Thus, this paper is the first report of chromosomal evidence of natural hybridization between these two taxa.

MATERIALS AND METHODS

In July 1990, a plant with features similar to *Cirsium altissimum* and *C. discolor*, yet phenotypically different from both taxa,

was observed growing in a depression on the roadside riverbank. This site was located at Nebraska City, Otoe County, Nebraska, seventy-five yards west of the Missouri River interstate bridge linking Iowa.

This plant was one of a few plants left intact after a recent brush-cut. It was observed that dried-up remnants of additional *Cirsium* plants formed part of the debris, but no other live *Cirsium* plants were observed in the vicinity of the putative hybrid site. However, on the Iowa side of the riverbank about thirty yards across the river obliquely opposite the hybrid site, a mixed population of nine *C. altissimum* and seven *C. discolor* plants grew. No plants phenotypically different from the two species were found at this site. The occurrence of a mixed *C. altissimum* and *C. discolor* population in such close proximity to the putative hybrid site led to speculation that the brush-cut *Cirsium* plants could have been members of either or both of these taxa and/or their derivatives.

The putative hybrid specimen was sampled in addition to specimens of the Iowa *Cirsium altissimum* and *C. discolor* mixed population. Two immature capitula and twenty-seven cypselae were collected from among aborted ovaries of mature capitula of the suspected hybrid plant. Seven immature capitula and fifty-six cypselae from three mature capitula were sampled from *C. altissimum*, and five immature capitula and thirty-three cypselae from two mature capitula were harvested from *C. discolor*. Capitula were bisected and fixed in a mixture of 100% ethyl alcohol and glacial acetic acid (3:1, V/V). Cypselae were germinated in a growth chamber to obtain root tips for chromosome counts.

Microsporocytes were stained according to the method of Feulgen-Rossenbach (1924), and root tips according to the method of Snow (1963). Chromosome spread was achieved by the application of a gentle but firm pressure on the stained specimens by rolling the thumb once over the coverslip. Chromosomes were photographed using an American Optical microscope-camera system with 5 × 97 ocular-objective combination.

Data for morphological comparison were obtained from measurements of nineteen characters (Table 1). The mean values of 10 measurements for each character of each plant were used. Voucher specimens (branches) of *Cirsium discolor*, *C. altissimum* and their putative hybrid are deposited at NEB.

Cirsium discolor: IOWA, Fremont Co., riverbank, 50 yards west of the Missouri River interstate bridge linking Nebraska City, 27 Jul 1990, *Dabydeen* 9001, 9002, 9003 (NEB).

Cirsium altissimum: IOWA, Fremont Co., riverbank, 50 yards west of the Missouri River interstate bridge linking Nebraska City, 27 Jul 1990, *Dabydeen* 9004, 9005, 9006 (NEB).

Cirsium altissimum × *Cirsium discolor*: NEBRASKA, Otoe Co., roadside riverbank, 75 yards west of the Missouri River interstate bridge linking Iowa, 27 Jul 1990, *Dabydeen* 9007 (NEB).

RESULTS

Cirsium altissimum has a chromosome number of $2n = 18$ (Figures 1a and 1f). In *C. discolor*, the chromosome complement is $2n = 20$ (Figures 1b and 1c). The suspected hybrid individual has a complement of 19 chromosomes (Figure 1d). All chromosome complements observed at mitotic metaphase are composed of metacentric to submetacentric chromosomes.

In most florets of the two immature capitula of the suspected hybrid, microsporogenesis had been completed already, and only a few anthers with microsporocytes at two stages of meiosis were found. Meiotic analysis of the putative hybrid specimens revealed that some of the bivalents were closely associated on an end-to-end basis and illustrated chain formation. In Figure 2g, a chain of six chromosomes, six bivalents, and one univalent (arrow) are shown. Microsporogenesis was not normal; at least one of the four microspore nuclei appeared relatively small (Figure 1e). Only three of twenty-seven (11%) cypselae of suspected hybrid origin germinated; cypselae of both *Cirsium altissimum* and *C. discolor* had 100% germination.

Measurements of nineteen characters (Table 1) of the suspected hybrid individual showed that fourteen were intermediate compared to the parental types. Three characters were approximately the same as either parental type, one character was larger, and one smaller than either of the parental types.

DISCUSSION

In interspecific hybridization events, hybrid phenotypes often express morphological intermediacy of many parental characters. In the suspected *Cirsium* hybrid specimen, fourteen characters, including four leaf features, were intermediate between those of

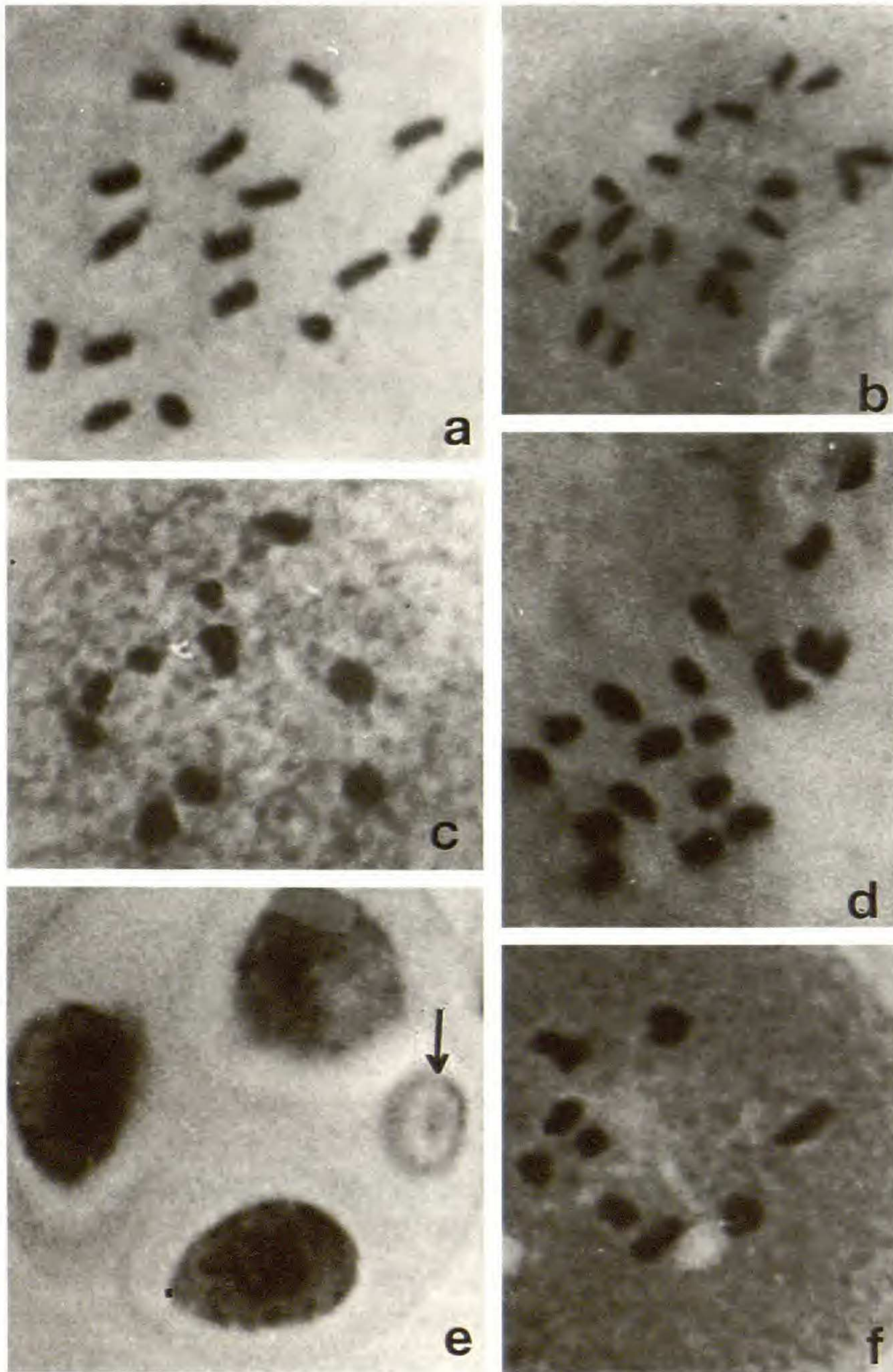


Figure 1. Photomicrographs of mitotic and meiotic chromosome complements of the genus *Cirsium*, X 800 unless otherwise stated. a. *C. altissimum*: mitotic metaphase, $2n = 18$. b. *C. discolor*: mitotic metaphase, $2n = 20$. c. *C. discolor* microsporocyte: metaphase I, 10 bivalents. d. *C. altissimum* \times *C. discolor*: mitotic metaphase, $2n = 19$. e. *C. altissimum* \times *C. discolor*: abnormal microsporogenesis and micronucleus (arrow) X 300. f. *C. altissimum* microsporocyte: metaphase I, 9 bivalents.

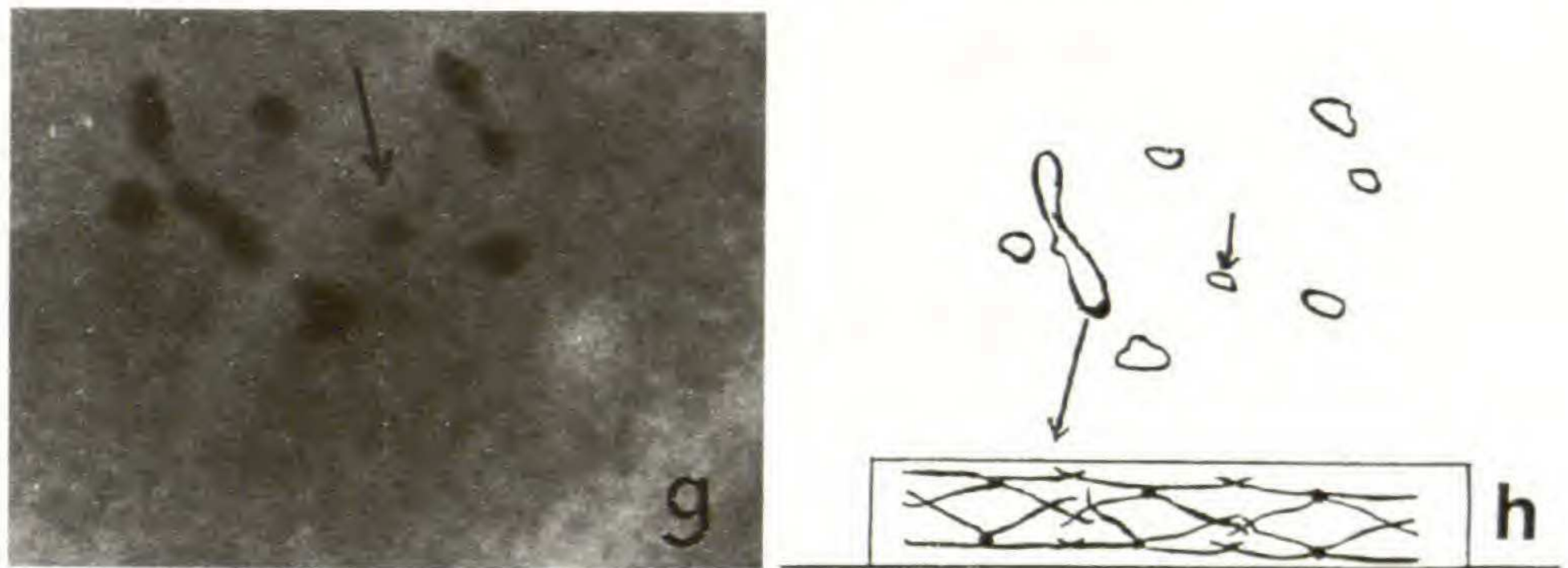


Figure 2. A photomicrograph ($\times 800$) and an interpretive drawing of the meiotic chromosome complement of the genus *Cirsium*. g. *C. altissimum* \times *C. discolor* microsporocyte: metaphase I showing 1 chain of 3 bivalents, 6 bivalents, 1 univalent (arrow). h. A drawing of the photomicrograph highlighting the chain to illustrate its formation through reciprocal translocations among the 3 bivalents (long arrow).

Table 1. Comparison of character measurements of *Cirsium altissimum*, *C. discolor*, and their hybrid derivative (all measurements in millimeters).

Characters	<i>C. altissimum</i>		<i>C. altissimum</i> \times <i>C. discolor</i>		<i>C. discolor</i>	
	Mean	S.E.	Mean	S.E.	Mean	S.E.
Involucre: length	29.7	0.48	28.2	0.85	26.8	1.01
width	21.6	0.85	22.3	0.85	23.7	1.03
Phyllaries: no. of rows	12.3	0.15	11.5	0.25	11.2	0.33
spine length	3.7	0.15	3.7	0.15	4.0	0.26
inner: length	26.1	0.53	24.3	0.47	22.4	0.70
inner: width	1.7	0.09	1.5	0.13	1.5	0.17
Pappus length	26.1	0.44	25.1	0.50	24.7	0.75
Corolla: lobe length	8.6	0.22	8.0	0.26	8.0	0.33
tube length	9.1	0.18	8.2	0.20	6.0	0.26
Stamen length	17.5	0.36	16.4	0.40	13.3	0.34
Stigma length	6.1	0.14	6.1	0.23	5.0	0.29
Style length	32.9	0.78	32.3	0.54	31.8	1.33
Leaf: length	100.8	3.78	103.4	1.96	112.5	3.57
width	18.4	1.30	16.4	0.62	15.4	0.62
lobe length	18.5	1.04	20.2	0.90	22.0	0.77
lobe base width	11.3	0.82	9.3	0.42	10.2	0.49
spine length	4.4	0.18	5.0	0.26	4.8	0.29
Internode length	22.3	1.27	21.1	1.20	20.6	1.11
Flower length	39.0	0.84	39.0	1.62	36.0	1.33

C. altissimum and *C. discolor* (Table 1). Ownbey (1951) investigated *C. discolor*, *C. altissimum*, and their putative hybrid derivatives and determined that plants with intermediate leaf types were hybrids.

The diploid chromosome complement of $2n = 18$ in *Cirsium altissimum* has been reported previously and the number is regarded as stable (Frankton and Moore 1963; Ownbey and Hsi 1963; Johnson 1974). In *C. discolor*, a diploid chromosome number of $2n = 20$ has been determined (Frankton and Moore 1963; Ownbey and Hsi 1963; Bloom 1977). In this taxon, accessory chromosomes have been occasionally observed, but in a closely related congener, *C. muticum*, frequent observations of extra chromosomes have been reported (Frankton and Moore 1963).

Accessory chromosomes usually do not exert phenotypic influence. Thus, based on morphological data (Table 1) and also cytological features (Figures 1d and 2g), it seems very likely that the nineteen chromosomes observed in the putative hybrid specimen are products of interspecific hybridization between *Cirsium altissimum* and *C. discolor*. These nineteen chromosomes, therefore, likely do not constitute a genotype of a *C. discolor* specimen with a supernumerary chromosome. It is probable that 10 chromosomes must have come from *C. discolor* and 9 chromosomes from *C. altissimum*.

The observation of a chain of six chromosomes, six bivalents, and one univalent (Figure 2g) during meiosis of suspected hybrid microsporocytes suggested that 9 chromosomes of *Cirsium altissimum* and 9 chromosomes of *C. discolor* are homologous or homeologous, and that reciprocal translocation is involved in the formation of the meiotic configuration. The univalent must have come from *C. discolor*. Similar cytological features (e.g., chain formation) were observed in microsporogenesis of interspecific hybrids of other *Cirsium* taxa (Dabydeen 1987; Bloom 1977).

The formation of micronuclei during microsporogenesis was probably due to chromosomal deficiency arising from meiotic aberration. Similar micronuclei formation observed in microsporogenesis of *Cirsium flodmanii* \times *C. undulatum* hybrids was attributed to lagging chromosomal materials (Dabydeen 1987).

The failure of many ovaries of the suspected hybrid plant to mature may be due to a lack of fertilization or some genetic aberration attributable to hybridity. There was no insect damage or any other external evidence of damage that would prevent fruit

formation. Many of the aborted ovaries were shriveled or extremely flattened. The extremely low rate of viability of cypselae may be due to hybridity. A high degree of sterility of *Cirsium* hybrids has been reported (Ownbey 1951, 1964; Bloom 1977; Dabydeen 1987). Bloom (1977) attributed the high degree of sterility of *Cirsium* hybrids to meiotic irregularities arising from chromosomal rearrangements.

The absence of hybrids at the site of the mixed population of *Cirsium discolor* and *C. altissimum* is not expected. Hybridization events have occurred in many instances where these two taxa grew in close proximity to one another (Ownbey 1964). Apparently, at this site, unknown factors operated to prevent hybridization and/or the establishment of hybrid progenies.

It is suggested that the rather small size and possible recent establishment of the mixed population may have contributed to the absence of hybrids. At the Nebraska site, it is highly possible that the brush-cut may have destroyed a well-established mixed population, including additional hybrids.

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